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ON THE "SICK" SOILS OF PORTO RICO.

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INTRODUCTION.

When a soil decreases in productivity, even after rich manuring, or when certain crops are dying a gradual death on a soil that formerly yielded abundant harvests, the soil is termed "tired" or "sick." Such phenomena were known long ago, but have only recently been explained; however, even now discussion on the subject is not closed, since various causes are evidently at work to bring on that condition. Two classes of soil sickness can be distinguished: One is caused by attacks of parasites on the roots, as e. g. Nematodes, while the other is caused by conditions not of a parasitic nature. It is this latter class of phenomena which will here be treated in regard to its occurrence in Porto Rico. Various kinds of diseased conditions can again be distinguished within this second class, an example of which is the difference of the behaviour of crops in "sick" soils. It may occur that the soil is sick with respect to a specific crop alone and not to other crops, which may sometimes depend upon the difference in depth to which the roots of different crops penetrate, or it may become "sick" for crops in general; it may further merely effect a decrease in yield or it may also cause a gradual death of the crops cultivated in it.

Such "sick" soils have sometimes been wrongly termed by farmers "worn out", and on the other hand, soils that had become deficient in one nutrient or another were wrongly termed "Sick." (a). These conditions must be well distinguished. A "worn out" or exhausted soil must, by a rational manuring, again bring satisfactory harvests, but a "sick" soil will not become more productive by that means. A "sick" condition can be produced by an abundance of fungi or microbes in soils. If much organic matter not previously rotted, such as tankage, blood meal, cottonseed meal or fresh animal dung, is introduced into a soil for a series of years, the con-

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(a) A Russian author asserted some years ago, that a clover sick soil may be cured by abundant manuring with phosphate. This was certainly no genuine case of soil sickness.

ditions are made favorable for an undue increase of microbes. (a) If, in such a case, the soil is not open and porous, but rather of a close, clayey texture, the normal respiration of the roots will gradually suffer, as the innumerable aerobic microbes absorb the oxygen of the air which enters very slowly into dense soils and the air present will be charged also with the carbonic acid produced by the respiration of these microbes. As a result the roots will not only be deprived of sufficient oxygen but they will also suffer from an excess of carbonic acid added to the carbonic acid produced by the respiration of the roots themselves. The bacterial life in the soil is mostly considered as being beneficial but there exist certainly cases in which the damage produced by it is greater than the benefit derived.

There exist anaerobic bacteria in the soil which can live at greater depth than the common aerobic soil microbes. Such facultative or obligate anaerobic microbes may cause fermentations which produce injurious consequences for the roots. As soon as a part of the root dies off (even under normal conditions some roothairs are continually dying off and new ones are produced) those microbes make a fresh start. By means of their enzyms they may sacharify starch and attack fine cellulose walls; they may not only feed upon these materials but also by fermentation turn them partly into injurious acids, as formic, acetic, or butyric. Naturally they will multiply most rapidly around the surface of dead and live roots and especially around the root hairs. Not only the acid producing microbes multiply when part of a root dies, but also such kinds as Proteus that yield alkaline compounds (as organic bases and ammonia) by decomposition of proteins contained in the roots. Among these basic compounds are poisons, as e. g. indol and scatol. Sometimes the conditions become suitable for microbes which, by reduction, change calcium sulphate to hydrogen sulphid and calcium carbonate. Hydrogen sulphid, which is also produced in the putrid fermentation of proteins, is a decisive poison for all living cells and even for the microbes themselves, although their power of resistance towards it is greater than that of other organisms.

These undesirable conditions will develop quicker in a close and moist soil than in an open porous soil, as the latter permits of a ready oxidation of injurious fermentative products by mold fungi and aerobic microbes. Nevertheless even in sandy soils "sickness"

(a) Organically manured soil may contain many millions of microbes in one cubic centimeter.

may appear when the ground water occupies continuously a high level or when cadavers of animals or roots of felled trees are left in the ground.

It has often been observed that well decomposed animal dung has a much better effect on the productiveness of a soil than the fresh dung, which fact is now well understood; on the one hand the injurious products of fermentation and putrefaction, either, have become more or less changed to harmless compounds, or were oxidized before the roots came into contact with them, while on the other hand the microbes that brought on the rotting in the compost heap have not had occasion to rob the roots of the limited supply of the oxygen in a close soil. Hence the hygienic conditions for the functions of the roots are much superior when well-rotted dung is applied than with fresh dung.

Tankage, bloodmeal, cottonseed meal (an excellent food for cattle) and other organic manures are often introduced in close clay soils although these material can become useful only after a complete rotting process which liberates the nitrogen of these manures as ammonia, sets free the phosphoric acid from the nucleo proteins, and turns the sulphid of the proteins into sulphate.

The old rule to apply organic manures one to six months before setting plants is doubtless a good one, but in tropical lands it would be still better to let them rot in a compost heap before introducing them into the soil, or to apply to a certain extent mineral manures.

Such a compost heap should, of course, be covered by a roof to prevent leaching by rains, further there should be added some gypsum and limestone meal besides earth, leaves, straw, or humus, etc., etc. It should not be kept too moist and should be aerated occasionally to enhance oxidation of injurious products formed by bacteria. A "dry rotting" or eremacausis is preferable to fermentations, the former being principally supported by mold fungi and aerobic microbes the latter by anaerobic microbes. Too much moisture may also cause denitrification, loss of nitrogen as gas. The products which protein matter, as tankage and dried blood, yield by oxidation are free of odor, consisting of carbonic acid, water and ammonia or nitric acid, the latter two being absorbed by the action of the gypsum and limestone meal respectivey. But the products which result from fermentation have a disagreeable odor, as for example of indol, scatol, butyric acid and hydrogen sulphid. The fermentation of proteins is commonly designated as putrefaction. Carbohydrates, as starch, hemicelluloses, pentosans, and the sugars can undergo various fermentations, which result in the production of

acids and in some cases also of various alcohols. The sugars ferment most readily. Roots containing sugar can, therefore, most easily give rise to acid fermentations in soils when they are allowed to die and rot in the soil after the plant is cut.

The process of eremacausis or dry rotting is most clearly exhibited in the decay of wood above ground. Here mold fungi are incessantly active to dissolve one constituent of the wood after another, utilizing them for respiration and growth. Often the wood is attacked by two or three different kinds of fungi at the same time, of which the one utilizes principally the starch, the other the lignin, and the third the cellulose, which remains after the lignin has been dissolved from the cellular walls. In such rotten wood the mycelia of the mold fungi gradually disappear again, being dissolved by their own enzyms. Aerobic microbes are capable, like mold fungi, of rapidly oxidizing the products of fermentation in soils brought on by facultative and obligate anaerobic microbes.

In recent years it has been found that "sick" soils can be regenerated by the application to the soil of carbon bisulphid and other disinfectants. (a) The investigations of Hiltner and Stoermer have shown that by this treatment the bacteria causing fermentations are killed first while various beneficial aerobic microbes survive partly and multiply rapidly after the bisulphid of carbon has gradually volatilized.

INVESTIGATION OF SICK SOILS

A high temperature throughout the year, combined with abundant moisture, leads to a luxuriant increase of bacterial life in soils when organic matter is present. Consequently, soils overcharged with bacteria will be more frequent in humid tropical countries than in cooler zones, where microbes cannot develop during the winter season. (b) It appears that in warm and humid climates those organic substances which otherwise cause the formation of humus are utilized by bacteria for their own development. On the other hand a certain amount of nitrogen is furnished by the ammonia of the abundant rains and by the activity of the nitrogen ga-

(a) Cf. Circular No. 11, Porto Rico Station.

(b) Erwin F. Smith, bacteriologist of the Bureau of Plant Industry, U. S. Department of Agriculture, arrived by his studies at the following conclusion: "The former idea that bacteria in general are not harmed by freezing is untenable. It was based on qualitative tests which are incapable of showing the true state of affairs in the exposed culture. Probably an enormous number of bacteria are destroyed every winter and those which survive come through in the form of endospores or some other resistant shape. These experiments confirm and extend those of Prudden Park, Sedgwick and Winslow."

therers Clostridium, Azotobacter, and B. Radicicola. The nitrogen content of the clay soils of Porto Rico thus far examined varies between 0.10 and 0.63 per cent, figures which appear rather high when the low content or even absence of humus in such soils is considered. This nitrogen content is probably due chiefly to living and dead microbes, mold fungi and infusoria.

Porto Rico (with exception of the southern district, which has an average of only 27 inches of rainfall annually) is provided with a great abundance of rainfall. The Mayaguez district has a rainfall of about 75 inches annually, and the eastern part of the island about 120 inches. It is no wonder, therefore, that such soils which are so frequently moistened harbor also great numbers of infusoria which feed upon the nutritive bacteria.

The test for infusoria is the same as that by which the presence of Azotobacter croococcum (a) is detected. This test is made as follows: A mixture of 1 per cent glucose solution with 2 per cent calcium carbonate, .1 per cent monopotassium phosphate, and .02 per cent magnesium sulphate is sterilized and then 10 to 12 per cent of soil is added. This mixture should remain in contact with plenty of air and hence occupy only a small volume in a flask provided with a cotton plug. Three to four days after adding the soil to the test solution great numbers of several kinds of infusoria can be seen under the microscope feeding upon the scum of Azotobacter cells which is developed upon the surface of the culture liquid. Infusoria have been found in soils of Porto Rico to a depth of 20 cm. below the surface. A small kind of amoeba has also been observed by this test.

Azotobacter (b) was found in every soil of Porto Rico that the writer has tested. It is not improbable that the nitrogen gatherer may show increased activity and enrich the soil more with nitrogen in the tropics than in northerly countries.

As to ''sick'' soil the inquiries made by the writer in different sections of Porto Rico have revealed soils sick for coffee, cane, pineapples, and for tobacco, and he did not fail to recommend treatment with carbon bisulphid which has given such excellent results in France and Germany.

Coffee sick soil from the Aurelia Plantation.—The proprietor

(a) While abundant access of air and the application of a relatively large amount of soil is necessary for the culture of Azotobacter, the conditions differ in the test for another nitrogen gatherer, Clostridium, inasmuch as access of air can here be restricted and even minute particles of soil may serve for successful inoculation.

(b) It may here be mentioned that the culture solution with manitol yielded purer cultures of Azotobacter than one with glucose

of the Aurelia plantation, which lies in hilly country about 11 kilometers east of Mayaguez, observed that the coffee trees on the higher portion of his plantation die a slow death which is not caused by parasites. The branches at first become yellow at the tip and the stratum of chlorophyll bearing cells below the surface of the bark turns brown, which phenomenon spreads gradually over the whole branch and kills it. The leaves of the diseased branches often show the incipient stage of the mosaic disease of tobacco; viz, a light green tissue between the veins and a dark green one along the veins, the leaves then gradually becoming yellow and brown and dropping off. The fruit of such trees turn red before ripening and rot later on or dry up. On a hill top which was divided from the sloping side by a trail the disease had made much progress, but on the slope below the trail the trees were not diseased.

The soil consists of a red clay in which no animal life was found except occasionally an earth worm. Nematodes could not be discovered by microscopical examination. It deserves special mention that the shade trees of this coffee plantation did not show any sign of disease except here and there an attack by a borer. The trees used for shade consist of some species of Inga, such as guamá and guava (leguminous plants serving generally as shade trees in Porto Rico) and further the center of the coffee sick portion contained a tree of Mangifera indica (mango).

The supposition of the owner that a deficiency of nutrients had caused the disease was not verified by his experiments. He had fertilized six plots, of 50 trees each, with tankage, dried blood, superphosphate and potassium chlorid, in various proportions. The result was, however, no essential change and even the best harvest on these plots did not approximate one half of the harvest on the unmanured healthy portion of the plantation.

The clay soil is of intense red color and gives a faint acid reaction. It was free of calcium carbonate (a). By extraction with warm water and evaporating the extract only traces of nitrate were found by the diphenylamin test, and by extraction with 1 per cent hydrochloric acid and testing with Nessler's reagent after neutralization only very faint traces of ammonia were observed. Upon heating in a test tube it turned black and evolved vapors of alkaline reaction and of an odor resembling that of decomposing proteins. This odor was more pronounced with the sick soil than with the

(a) Calcium carbonate causes in northerly countries often a decrease of humus, but here in the tropics no accumulation of humus takes place in absence of it. Only a prolonged inundation of land is here favorable for humus accumulation.

healthy soil. The "sick", dried soil, contained .34 per cent of nitrogen, which was probably chiefly present in the form of proteins stored up in unavailable form in the bodies of the bacteria. The humus content was determined by the usual method of extracting with dilute ammonia after the soil had been treated with dilute hydrochloric acid. The amount of this humus which may have included some protein extracted from the bacterial bodies by the ammonia, was found to be .91 per cent. In comparing this figure with the relatively high nitrogen content of the soil the inference that the bacteria must have had some ready source of nitrogen to build up their bodies may seem justified. Indeed the bacteriological tests have revealed, besides Azotobacter, also the nitrogen gatherer and butyric ferment "Clostridium pastorianum" in abundance. Even very small quantities of the sick soil after being in a glucose culture solution for two or three days produced butyric fermentation and developed the characteristic forms of that microbe, bearing a spore in the central widened part.

Sick coffee trees on the Rosita plantation, a short distance east of Mayaguez, showed phenomena different from those at the Aurelia plantation, making a genuine infection probable. Above all the guava shade trees are here also suffering and the owner has observed that they are attacked first, one branch after the other dying off, and that later on the coffee trees next to them also become sick, the base of the trunk showing a gradual softening. The attacked portions of the roots are black in color and under the microscope such softened portions show numerous fat globules, probably remnants of a fungus,the mycelium of which had dissappeared by being dissolved. It may be stated that the soil of this plantation otherwise much resembles that of Aurelia. In order to show the extent to which a butyric ferment was here attached to the dead roots the following test is mentioned: A small piece of dead root, weighing about 20 mgms., separated under aseptical conditions, was dropped in 8 c.c. of nitrogen free glucose culture solution (a). After 5 days at 20° to 28° C the entire wall of the test tube was coated with a film, which proved to be a pure culture of a large bacillus free of spores. The bottom of the tube showed flocculi of bacilli, developing gas bubbles and a strong odor of butyric acid.

(a) This culture solution, often used in this investigation contained 1 per cent pure glucose, 0.2 per cent dipotassium phosphate and 0.02 per cent magnesium sulphate. In this nitrogen-free solution the butyric bacilli can develop moderately until a certain degree of acidity is reached This solution is useful insofar as the great majority of other soil microbes cannot develop in it, and thus the search for the butyric microbe is facilitated.

The disease observed on this plantation was studied by the plant pathologist who found a fungus attacking the roots.

Soil sickness may, of course, be caused by an excess of very different species of fermentative bacteria. In cases, however, in which an undue excess of the butyric ferment caused that condition, the application of bisulphid of carbon would probably have to be repeated after a short time on account of the spore production of that microbe and the resistant power of the spores toward poisons. However, the writer is not yet sure that the soil sickness of Aurelia is exclusively due to an abundance of that microbe, which in the struggle for existance with many other kinds of bacteria, has the advantage of being capable of exerting its activity in the absence of oxygen and in the deficiency of nitrogen compounds.

This microbe produces a special fermentation of sugar, changing it to butyric and acetic acids and certain alcohols with development of carbonic acid and hydrogen. The dead roots left in the ground often contain sugar or the easily saccharifiable starch. Taking these facts into consideration it cannot be denied that an unhealthy condition may be produced for the live roots, as the acids mentioned kill the root hairs which are so necessary for absorbing the mineral nutrients of the soil. This danger is especially great in soils free of calcium carbonate and giving a weak acid reaction, since the acids are not neutralized and are only gradually removed by oxidation. Equal numbers of the butyric microbes may cause much less damage in soils containing a moderate amount of carbonate of lime. The butyric microbes are very common. The writer found it in Porto Rico in the dust of a room, on the surface of insects, on leaves of trees and in wellwater. It was found in soils to a depth of 60 cm., while at 85 cm. depth it was not present in all samples.

It was found absent on a blade of grass from the middle of a meadow exposed the whole day to the direct rays of the sun and also in a sample of bat guano from a cave near Aguadilla. Our butyric microbe behaves like a facultative anaerobic microbe and can easily be recognized by its spore bearing form as well as by the butyric fermentation it produces in glucose culture solution free of nitrogen compounds.

A special experiment was made on the action of bisulphid of carbon on the sick soil of Aurelia. One hundred grams of soil and 8 c.c. of bisulphid of carbon were put into a wide mouth bottle which was well closed and let stand for three weeks. The examination showed that only spore-forming kinds had survived, as B. butyricus,

B. subtilis, and B. mycoides. The view has been entertained by some authors, that the beneficial action of bisulphid of carbon on sick soils is due to its partial oxidation to sulphuric and carbonic acid, the former unlocking nutrients, but in our experiment no trace of sulphuric acid was formed.

Comparisons were made as to the kinds of bacteria present in sick and in healthy soils of Aurelia. Sterilized solutions 10 c.c. each of various compositions were infected with small doses of the soil, increasing in quantity to about the size of a pea. In such cases, in which the access of much air was desirable, as e. g. in the test for Bacillus methylicus, the solution formed a thin stratum in a conical flask plugged with cotton. Bacillus methylicus, an aerobic, harmless and universally spread microbe, was found more abundant in the sick than in the healthy soil, when a culture solution, in which the only organic matter was 0.5 per cent sodium formate was inoculated with these soils.

In order to test for the presence of microbes capable of decomposing nitrates with development of free nitrogen, thus causing a loss of valuable nutrient material, 0.3 gms. of healthy and of sick soil was added to a culture solution containing 2 per cent glucose and 0.1 per cent potassium nitrate. This mixture was kept in a small gas tube apparatus at a temperature of 32° C. After three days 4.1 c.c. gas was developed from the healthy soil and 6.2 c.c. from the sick soil. This gas consisted up to 50 per cent of carbonic acid with the healthy and 38 per cent with the sick soil. The further examination showed that butyric fermentation had developed which yields besides carbonic acid also hydrogen gas according to the following general equation:

$$\underbrace{C_6 H_{12} O_6}_{\text{Glucose.}} = 2 H_2 \div 2 CO_2 \div \underbrace{C_4 H_8 O_2}_{\text{Butyric acid.}}$$

Hence, therefore, the rest of the gas contained in the gas tubes must have consisted either wholly or to the greater part of hydrogen while free nitrogen could have been developed only to a small extent and that only with the sick soil.

A further test for such injurious denitrifying microbes was carried on with a culture solution containing 0.1 per cent pepton, 0.1 per cent sodium nitrate and 2 per cent ethyl alcohol but after three days only 0.85 c.c. gas had developed with the sick soil and 0.50 c.c. with the healthy one, 2 grams of soil (after substraction of moisture) having been applied in each case. That small amount of gas developed consisted partly of carbonic acid. It is therefore not

probable, that denitrification is the main cause of injury in this sick soil.

In order to test for microbes capable of reducing sulphates to hydrogen sulphide the following solution was inoculated with equal amounts (0.3 gm. to 10 c.c. liquid in narrow tubes) of both sick and healthy soils:

Ethyl alcohol	2	per cent
Ammonium sulphate	0.1	" "
Dipotassium phosphate	0.1	" "
Magnesium sulphate	0.2	" "

There was some bacterial developemnt in both cases, but more with the sick soil than with the healthy. After three weeks standing a strong odor of hydrogen was observed in the culture of sick soil, but not in that with the healthy soil, which points to the possibility of injury by the sulphate reducing bacilli in the sick soil, since hydrogen sulphide is poison for roots.

In order to test for yeast cells the following solution contained in test tubes was infected with small doses respectively of healthy and sick soil:

Glucose	5.0	per cent
Peptone	0.2	" "
Magnesium sulphate	0.1	" "
Monopotassium phosphate	0.2	" "

After two days turbidity and gas development had appeared with the sick soil, but not with the healthy. After two days more the six tubes containing sick soil showed a froth of one half centimeter in height and a lively gas development, while the healthy soil had just commenced to develop gas and no froth whatever was visible on the surface. Microscopical examination showed that the fermentation was not the alcoholic fermentation caused by yeast, but the butyric caused by the butyric bacillus Clostridium, as the odor of the liquid had already led us to suspect. (a)

In the test for mold fungi the solution just mentioned was diluted four times and then .5 per cent tartaric acid was added. In this acid solution bacteria did not develop at all neither after inoculation with the healthy nor with the sick soil, but some mycelium did which later on also showed spores. Here as well as in the other cases the kinds of mold fungi differed frequently after inoculation

(a) Only one of the tubes contained some distorted forms of yeast cells, which resembled most closely certain kinds of Torula; it had formed a surface film on one of the tubes with healthy soil.

with sick and with healthy soil, which may have been accidental. Some of the fungi seemed to belong to species of Penicillium. The colors of the spores were either green, gray, black or yellow.

A further comparison was made with regard to the number of microbes developing under aerobic and anaerobic conditions. In the former case gelatin plates were used, being kept at 22° C., while in the latter plates of glucose agar were used which were kept at 32° C. Thus the interesting fact was ascertained that at about one foot depth there existed more aerobic microbes in the healthy soil, while there was an unusual excess of anaerobic microbes in the sick soil. The aerobic test from two determinations yielded the following number of bacilli in one gram of soil:

Healthy soil	914,500
Sick Soil	774,000

From the character of the colonies the presence of Bacillus mycoides, B. proteus, B. subtilis, B. anthracoides and B. fluorescens liquefaciens was easily established. The prevalence of B. mycoides was especially noticeable, forming 86 per cent of all the colonies on the plate of sick soil, while on the plate of healthy soil it formed only 49.2 per cent. Next in number came B. proteus followed by B. subtilis, while B. fluorescens was present only in a small number.

The anaerobic plates were prepared after Stuelers method. The anaerobic bacteria found per gram of soil were as follows:

Healthy soil	3,295
Sick soil	10,807,020

These anaerobic colonies resembled each other so much that they seemed to belong to one and the same species. The spore bearing form and the power to produce readily butyric fermentation in glucose culture solution left no doubt that this microbe present in such excessive numbers was the butyric ferment (Clostridium) or a variety of it. The anaerobic condition, however, is not absolutely required for its development on the plate, but the presence of glucose or of related fermentable material is necessary.

The greater content of anaerobic microbes, in the sick soil agrees with the findings of the chemical analysis showing 0.34 per cent nitrogen in the sick soil against 0.13 per cent in the healthy soil.

It is, moreover, an interesting fact that the mechanical condition of the soil was somewhat altered by the excess of bacterial life, inasmuch as the amount of colloidal clay in the sick soil had de-

creased. The supposition suggests itself that certain bacterial products had cemented together the finer clay particles, thus preventing the colloidal state. When the healthy and the sick soil were shaken with 10 times their amounts of distilled water, the supernatant liquid became clear in a few hours with the sick soil, while it was still very turbid even after two days with the healthy soil. It may also be stated here that the sick soil contained traces of fatty matter (a) whereas the healthy soil did not.

The healthy and the sick soil of Aurelia were geologically and mineralogically identical and also in a chemical respect the differences were not such as to allow any interference in regard to the different behavior towards vegetation. We add here for comparisons sake the chemical analyses, furnished by the chemist of the station:

Chemical composition of healthy and sick soils of Aurelia.

	Healthy soil.	Tired soil.
Insoluble in hydrochloric acid	52.01	46.13
Soluble in hydrochloric acid of 10%	13.76	18.60
Fe_2O_3	13.07	11.58
Al_2O_3	20.71	23.73
CaO	0.23	0.32
MgO	0.07	0.65
K_2O	0.13	0.06
P_2O_5	0 09	0.08
Moisture	4.45	8.70

Coffee sick soil of the College Plantation.—At the College Plantation, about a mile north of Mayaguez very similar conditions were observed as at the Aurelia plantation. The soil also consists of a red clay and the coffee trees growing on certain parts of that farm also die a slow death in absence of any parasites. Small particles of the dead roots or of adhering soil when put into a glucose culture solution also produced a much more powerful butyric fermentation within 2 to 3 days than equal amounts of root or soil from the healthy part of the plantation. The sick soil is somewhat darker and contains less colloidal clay than the healthy soil nearby, recalling the differences existing also at Aurelia. Five grams each of air dry healthy and sick soil, of the College plantation, were shaken in a finely divided state with 50 c.c. distilled water and the mixture was left to settle in a large test tube. After 20 hours 25 c.c. of the turbid liquid above the sediment was pipetted off and evaporated

(a) By extraction with ether containing some alcohol nine grams of sick soil yielded 5 milligrams of a yellow fatty substance, different from agroceric acid.

in a platinum dish. Thus 0.136 grams was obtained from sick soil while 0.263 grams was derived from the healthy soil, corresponding to the relative figures of 5.44 per cent and 10.52 per cent of colloidal clay. These residues were carefully extracted by 0.2 per cent hydrochloric acid; a clear solution of this extract yielded a stronger reaction for sulphates with the healthy soil than with the sick soil.

The odor of the sick soil was moldy, that of the healthy soil, however, not. On heating in a test tube, the sick soil developed strongly alkaline vapors, the vapors produced by healthy soil were much weaker.

The determinations of the numbers of aerobic microbes developing on gelatin plates was difficult since B. mycoides developed in such large numbers and with such rapidity that by its liquifying the gelatin the further development of various other colonies that would otherwise have developed, was entirely prevented. This circumstance led me to kill off with nitrate of silver all the colonies of B. mycoides developed after 24 hours on the gelatin plate and again further colonies of this microbe developed 20 hours later on, and thus a great number of colonies of other microbes were able to develop. Thus it was found that there was not an essential difference between the number of aerobic microbes in the healthy and the sick soil, as an average of two determinations with two different samples, showed 210,000 aerobic microbes in the sick and 265,000 in the healthy soil.

The determinations of the number of anaerobic microbes did not lead to perfectly satisfactory results here; many of the spores of the butyric bacillus present did evidently not develop on the sugar-agar plate at 34° C. This difficulty had already been observed by others. Migula in his System der Bacterien mentions that certain varieties of the butyric bacillus do not develop colonies on a dry culture medium. At any rate there were conditions in the soil of the former plantation which differed from those at Aurelia in this regard, although the sick soils in both cases produced, in sugar solutions, a much more powerful butyric fermentation within 3 to 4 days than was the case in healthy soils.

The tests for sulphate reducing microbes showed that they were present in the healthy as well as in the sick soil; the writer applied ethyl alcohol to differentiate the microbial life in favor of these bacteria, since the relatively high hydrogen content of that alcohol seemed to be especially adapted for the reduction of sulphates to hydrogen sulphid by the specific microbes. This supposition was confirmed by the experiments and the writer is therefore of the opinion that this new and simple method may often prove to be

valuable (a). The solution applied had the following composition:

Ethyl alcohol	2	per cent
Sodium acetate	0.5	" "
Asparagin	0.1	" "
Dipotassium phosphate	0.2	" "
Magnesium sulphate	0.02	" "

This solution was applied with and without addition of gypsum and the result showed that the presence of this sulphate accelerates the formation of hydrogen sulphid considerably. Two grams of the finely crushed soil was mixed with 0.5 grams of gypsum and 20 c.c. of the above solution and the mixture kept in a large test tube at 34° C. Strips of filter paper moistened moderately with basic lead acetate were hung in the test tube which was closed with a cotton stopper. After two days blackening of the lead paper was observed, hence, hydrogen sulphid had been generated in both cases. In the absence of gypsum a weak blackening appeared 4 days later.

It is worthy of further mention that in both test tubes with healthy soil the surface of the soil sediment became black from the formation of sulphid of iron. Since this was not the case in both test tubes with sick soil, the inference might be drawn that the ferric oxid was present in the former in a more easily reacting condition. If this would prove correct, a more available condition of the iron for the roots would exist in the healthy soil.

Cane sick soil from Constancia.—There exists a considerable tract of loamy soil in the San Germán valley which, without manuring, has served successfully for cane culture for probably more than 150 years. At the present day it is considered as "worn out." The average production is now estimated at 17 tons; the yield in 1908 was only 12 tons of cane per acre. In the past three years the land had been manured with 400 pounds of fertilizer per acre containing 9 per cent nitrogen, 8 per cent potassa, and 7 per cent phosphoric acid, but the conditions were not improved thereby. The last manuring consisted of 400 pounds of tankage per acre, but the harvest has not yet been determined. Since parasites have not been discovered, a true case of soil sickness is here very probable and disinfecting experiments were carried on four weeks before the new cane was planted.

(a) Thus far only Beijerinck (Centr. Bct. 1985 p. 1) and Delden (Ibid. 1904, p. 81 and 113) have published investigations on sulphate reducing microbes, which they isolated from the mud deposits in ponds. These "spirilli" were named Microspira desulfuricans and Microspira des tuarii, the latter occurring in salt water; both were found strictly anaerobic. These authors applied malates and similar salts in the culture solutions.

Not far from this locality, at the Hacienda San Francisco, close to Hormigueros, the observation was reported by an official of the Guanica Centrale that the young cane made an exceptionally slow growth compared with other cane cultures in the vicinity. In this case not only an abundance of the butyric ferment but also a parasite (Marasmius sacchari) was discovered by the plant pathologist of the station. Mineral fertilizers would certainly be preferable in such soils overcharged with bacteria and fungi. Indeed at the cane plantation of Los Caños, where the nitrogen is provided in the form of sodium nitrate and ammoninum sulphate, no such troubles have been reported. Fertilizer was applied to the amount of 600 pounds per acre, as top dressing of a mixture of

100 pounds of potassium sulphate.....	Containing
100 pounds of ammonium sulphate.....	12.52 per cent
200 pounds of sodium nitrate.........	Nitrogen

The cane leaves which were heaped up between the rows of cane and plowed under after the harvest were first exposed to a dry rotting process.

Near Hormigueros the fertilizing effect of tankage with a 12 per cent nitrogen content and 7 per cent bone phosphate, was compared with that of ammonium sulphate (a) and sodium nitrate. An employee of the Guanica Centrale, carried on this experiment on plots of one tenth of an acre, from which he obtained the following results per plot:

	At Constancia Pounds	At Hacienda Luisa Pounds
Tankage 300 lbs.	4,910	4,640
Tankage 400 lbs.	6,490	4,870
Ammonium sulphate 300 lbs.	6,800	5,900
Ammonium sulphate 400 lbs.	8,065	5,690
Sodium Nitrate 300 lbs.	6,600	5,790
Sodium Nitrate 400 lbs.	7,185	5,185
Control plot	5,920	4,300
Control plot	5,325	3,825

Cane sick soil from Las Dolores Plantation.—The proprietor of this plantation which is in the vicinity of Añasco, reported that the young cane developed only to a height of 30 to 40 cm., then stopped growth and died gradually, whereupon a new shoot would start which also died off some time afterwards. No healthy growth was

(a) Attention must be paid to the time of application as ammonium sulphate should not be applied too late or in too large a quantity, otherwise more leaf and less sugar will result.

possible and that same field has now been left to grass for two years. Here as well as in the case of soil from the Constancia sugar plantation a great abundance of the butyric microbes was observed. An odor of butyric acid with turbidity, and gas developed within a few days when particles of soil, the size of a pin's head, were transferred under sterile conditions from the interior of a large lump of soil into a narrow test tube containing 5 ccs. of a 1 per cent nitrogen free glucose solution. The microscope then revealed the spore bearing forms of Clostridium pastorianum accompanied by a long motile bacillus bearing a bright globule at one end.

When this test was modified with the soil of Constancia for Azotobacter culture, the glucose solution was kept in a conical flask with access of an abundance of filtered air and the infection was made with 12 grams of soil for 100 c.c. liquid, in presence of some calcium carbonate. A rich scum of Azotobacter with accompanying infusoria appeared in four days at 25° to 30° C. After nine days an odor of hydrogen sulphid developed, lead paper was blackened by the air in the flask, and the soil assumed a black color from the formation of sulphid of iron. The microscope now showed various forms of microbes, among them a small spirillum of lively motion which probably subsisted on the Azotobacter cells first developed. Whether this hydrogen sulphid was produced by decomposition of proteins formed by the Azotobacter growth, or whether it was due to a direct reduction of sulphate, could not be directly determined. The writer, is, however, inclined to assume the latter possibility. A sandy soil from Peña Cortada near the sea beach was here used for comparison and yielded no such development of hydrogen sulphid under similar conditions.

In order to determine whether that soil contained bacteria able to reduce sulphate to hydrogen sulphid a sample of soil was added to the following solution:

Water	100
Ethyl alcohol	2.
Ammonium sulphate	0.1
Magnesium sulphate	0.1
Dipotassium sulphate	0.1

In this solution, first turbidity, and after ten days a strong odor of hydrogen sulphid was developed and lead paper turned intensely black when hung into the flask. Hence, reduction of sulphate had been accomplished. In a second test a culture solution having the following composition was used:

Sodium acetate	0.5	per cent
Ethyl alcohol	2.0	" "
Asparagin	0.1	" "
Calcium sulphate	0.2	" "
Dipotassium phosphate	0.2	" "
Magnesium sulphate	0.02	" "

Constancia soil 5 grams to 20 c.c. solution produced again in the course of 10 days considerable hydrogen sulphid. Aerobic plates of Constancia soil were prepared with gelatin and with sugar gelatin. The result calculated for one gram dry soil was as follows:

On gelatin	138,000 colonies
On sugar gelatin	1,179,100 colonies

The latter were chiefly butyric bacilli. Hence, the microbes producing fermentations surpassed the common aerobic ones about eight fold. The colonies of B. mycoides formed 3.3 per cent of the total colonies on sugar gelatin and 4.1 per cent on the common bouillon gelatin; B. subtilis colonies were fewer and ranged next in number, then followed Proteus communis and B. anthracoides and finally B. fluorescens liquefaciens. There were also colonies present resembling in character those of B. coli and such as resembled those of B. lactis aerogenes and others.

Tobacco sick soil of Caguas.—There exists on the Rio Grande and on Buena Vista farms much land used for tobacco culture, but about 75 per cent of that land gave very poor results. The upper land especially gave such low returns that it did not pay to continue this culture. For three years tobacco has been tried and the land had been manured with cottonseed meal at the rate of 1,000 to 2,000 pounds per acre. On the Buena Vista farm which was especially well manured with cottonseed meal, the tobacco plants died a slow death when one to two feet high. Thirty acres of this farm was planted two years ago and last year is was increased to 50 acres. Not far away lies the Amor plantation which yielded such poor results that it was sold again by the tobacco company. Inquiries by the writer showed that all these loamy lands, not responding even to heavy manuring, had been formerly cane land and had served for this culture about 150 years or over. Other farms near Cayey that had formerly not been used for cane culture gave very satisfactory harvests of tobacco after being manured with cottonseed meal.

Special care should be exerted in the selection of manure for lands which have served for cane culture for a long period of years. Since the roots of cane contain about five per cent of the total sugar

of cane, and since these roots are frequently left to rot in the ground, the increase of microbes, among which may be injurious ones also, will be exceptionally large in these soils, and if, under such conditions, organic manures such as cottonseed meal, are applied the microbes will feed upon it and multiply before the roots can utilize its nitrogen, phosphoric acid and potassa. When such lands are used for tobacco mineral manures should be chiefly employed while organic manures should be used only after being well decayed. Tobacco requires quickly acting manures, the harvest being made six weeks after setting out the plants.

The bacteriological tests have shown the butyric ferment to be present in these sick soils in great abundance.

Soils sick for tobacco exist to the extent of several acres also on the Jobo farm, 15 kilometers south of Arecibo. Various manures and crops besides tobacco have been tried without producing any satisfactory growth. When the supposition that probably the decaying roots of trees grown on that land caused that condition was mentioned to the proprietor, he expressed the opinion that the roots of the Pomarosa trees (a) occurring abundantly in the thickets of Porto Rico, probably were the cause of such bad conditions. Near those decaying roots of the tree no growth of other plants was possible. This root probably contains much starch or other carbohydrates; it is very large and so difficult to dig out that the farmers hesitate to incur the expense of removing it.

Some experiments formerly made by Daikahara (b) have shown that even small amounts of starch added to soils may depress the harvest to some extent by favoring injurious fermentations. An amount of 10 grams starch added to 11 kilo humy loam soil depressed the yield in rice per pot from 25.50 to 23.25 grams and the yield of buckwheat from 21.88 to 16.75 grams (average of three experiments).

A garden soil was infected with cultures of the butyric bacillus in bouillon and to a part of this soil 2 per cent of starch was added. Five branches of Tradescantia were planted to each pot. After four weeks the total height in the latter case had increased 48 per cent and in the absence of starch 71 per cent.

(a) Jambosa jambos.

(b) Bulletin, Experiment Station Nishigahara, Tokyo, vol. 1, No. 2, pp. 31-33.

FINAL REMARKS.

The sick soils examined show, in comparison with healthy soils, a notable abundance of the butyric microbe. In three cases of sick soil microbes were observed which were able to change sulphates into the poisonous hydrogen sulphid. Denitrification microbes causing a loss of nitrogen from nitrates in the soil do not seem to play an important role in the sick soils.

Since the butyric microbe occurs widely spread in all soils, special care should be taken not to permit it to increase excessively by allowing roots to decay in the ground as decaying roots form a suitable nutrient for that microbe, especially when they are rich in sugar and starch. The cellular walls of decaying roots may be opened and eaten away by cellulose dissolving mycelia. This latter process opens the way for the butyric microbe which feeds upon the cell contents. This microbe may on the one hand injure the new root hairs of living roots by the acids and enzyms it produces and on the other hand it has the power to deposit much nitrogen in its spores, which remain unaltered in the soil a long time, thus withdrawing much nitrogen from circulation.

The butyric bacillus has the power to utilize the free nitrogen of the air and to transform it into proteins of its cells, hence it was looked upon as a means of enriching the soil with nitrogen compounds. But an undue increase of that microbe can do more harm than good.

Since the disinfection of the soil with bisulphid of carbon, which has given such excellent results in orchards and vineyards of Europe, may become too expensive in certain cases the question arises whether cheaper methods of disinfection are not available for cane plantations. In this regard attention may be called to the fact that many kinds of microbes are readily killed by the direct rays of the sun. This principle of disinfection may be of good service where land is plowed with the steam plow three to four times at various certain intervals. The land should be plowed very deep so as to expose new surface of soil to the sun rays (a) at every plowing.

Attention should be paid to exclude fermentations from the soil. Fermentations can be caused by certain microbes (b) which can develop even in absence of air. A lack of aeration will diminish

(a) By this means also the roots are gradually removed. If the sugar of the cane roots can be profitably extracted, two advantages would be gained by removing the cane roots from the soil.

(b) Fermentation caused by yeast will probably not play any role in the ground. Yeast cells belong to a higher group of fungi than do microbes.

or exclude the common aerobic microbes which oxidize organic materials in the soils and may, in this respect, serve a useful purpose. Such a deficiency of aeration and presence of fermentable materials form, on the other hand, favorable conditions for facultative or strictly anaerobic microbes which often produce injurious fermentations. Hence provide a healthy home for the roots, they will repay it in the form of full harvests.